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Docking Studies of Pyrazole Derivative as Anti-Inflammatory Drug With Cyclooxgenase-2

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ABSTRACT:
The key feature of inflammation is pain and it is mediated by molecules called prostaglandin, a prostanoid. Cyclooxygnase (Cox) is an enzyme responsible for the formation of prostanoids. There are two types of Cox. Cox1 is found in nearly all tissues whereas Cox2 is up regulated only in inflammatory tissues. Thus the inhibitors of Cox2 can be targeted to treat inflammatory diseases. Celecoxib is one of the Cox2 inhibitors and it belongs to NSAIDS class. They reduce inflammation without affecting digestive system. It also suppress the tumor growth. These pharmacological effects enabled to design an analog of celecoxib. The analog was designed in such a way that it led to a 70% decrease in Cox2 metabolism, but retains the inhibiting properties of the original ligand, celecoxib. The aim of the present investigation is to predict the potency and activity of analog using both MAESTRO &GLIDE (Grid based ligand docking), a tool for drug design. This study will be useful to target various inflammatory diseases like arthritis, Alzheimer's and also the cancer.

Key words: Cyclooxygenase, NSAID, Docking, Celecoxib, Inflammation, Prostaglandins.

INTRODUCTION

Inflammation is a basic way in which the body reacts to infection, irritation or other injury. The key feature being redness, warmth and sensitive nerve endings in our body become irritated. It is mediated by molecules called prostaglandins, a prostanoid.

Cyclooxygenase (Cox) is an enzyme (E.C.1.14.99.1) that is responsible for formation of important biological mediators called prostanoids pharmacological inhibition of Cox can provide relief from the symptoms of inflammation and pain. Currently there are three Cox iso enzymes are known namely Cox1, Cox2 & Cox3. Cox1 is a constitutive enzyme and is found in almost all cell of the body except red cells. The Cox 2 enzyme is located specifically in inflammatory areas and it is inducible [1-5]. Cox 3 is a splice variant of Cox 1 which retains intron one and has a frameshift mutation, thus some prefer the name Cox 1b or cox1 variant. Besides, Cox has long been recognized to be involved in normal kidney function [6-8], regulating brain function[9-11], maintaining proper glandular architecture of small intestine [12, 13], ovulation[14], Uterus contraction [15] and stimulate bone resorption & formation [16,17] Cox2 inhibitors are a new family of drugs that belongs to the class of Non-steroidal anti- inflammatory drugs (NSAIDS). They achieve inflammation reduction by selectively blocking the Cox 2 enzyme which obstructs the production of the prostaglandin that cause the pain and swelling. They selectively block Cox 2 not Cox1 and they reduce inflammation without affecting the normal function of the stomach or intestinal tract.

Celecoxib is an NSAID that is used to treat arthritis, pain, menstrual cramps, colonic polyps and advanced cancers. The pharmacological effects of celecoxib enabled the synthesis of close structural analogs of celecoxib that exhibited increased anti tumor potency in the absence of Cox-2 inhibition.

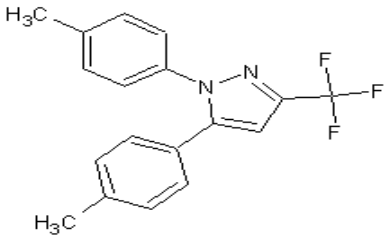

Fig.1. 1, 5-Bis-(4-methylphenyl)-3-trifluro methyl-pyrazole

1,5-Bis-(4-methylphenyl)-3-trifluromethyl-pyrazole (fig.1) is an analog of celecoxib. In this drug the sulphonamide group of celecoxib was replaced with methyl group. This modification led to a 70% decrease in the metabolism of Cox 2. The aim of the present investigation is to predict the potency and activity of celecoxib analog using GLIDE (Grid based ligand Docking with Energetics) a tool for drug design. It was also aimed to predict the feasibility of the compound as a lead for drug development, so that it will be useful to target various diseases like arthritis, alzheimer's disease ,Parkinson disease and various types of cancer which involves Cox inhibition for their treatment.

MATERIALS & METHODS

System configuration & software:

Pentium 4-3. 20 GHZ, 512mb of RAM, 40 GB hard disk drive, 1MB cache, 1.44" floppy disc drive, 17" color monitor, 128 MB AGP card. OS-LINUX EL – 4.0, Docking software GLIDE, visualization tool-pymol.

Docking analysis using Maestro

Maestro is the graphical uses interface for all of Schrödinger's products. Combiglide ™, Epik ™ Glide ™ impact ™, Liaison ™ ligprep ™ Macro model ™, phase ™, prime ™ qikprop ™ qsite ™ and strike ™. It contains tool for building, displaying and manipulating chemicals structures, for organizing, loading and

storing these structures and associated data and for setting up, monitoring and visualizing the results of calculations on these structures.

Protein preparation:
The protein structure (3D) was taken from PDB. The structure was refined using amber force filed and the model was taken as starting structure for the docking studies.

Ligand preparation:
All small ligand structures were drawn using Schrödinger soft wares builder panel, suberoyl anilide hydroxamic acid (SAHA) structure from PDB was taken as scaffold. The structures were then energy minimized using the OPLS-2005 force field until it reaches the RMSD 0.001 Kcal/mole

Glide Docking Method
Glide uses a hierarchical series of filters to search for possible location of the ligand in the active site region of the receptor. The shape and properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses. Conformational flexibility is handled in glide by an extensive conformational search, augmented by a heuristic screen that rapidly eliminates unsuitable conformation, such as conformations that have long range internal hydrogen bonds.

Two types of docking algorithm were used namely induced fit docking and high through put virtual screening. In the present study, induced fit docking was done as follows: 1) import the protein (4cox) from PDB, 2) all the water molecules are deleted 3) adjust the ligand bond orders and formal charges, 4) one ligand exist in two active site of the protein 5) docking was done, targeting the active site by keeping the appropriate ligand as reference and 6) run protein preparation.

RESULTS AND DISCUSSION
Table 1 shows the induced fit docking score table of original ligand. From the table, it was observed that the least docking score for the original ligand (celecoxib) was found to be -6.050 and the corresponding glide energy was found to be – 39.573Kcal/mol.

Table 2. Shows the induced fit docking score for new ligand. It was identified that the least docking score for the new ligand (pyrazole derivative) was – 3.4 and its glide energy was found to be -17.679 kcal/mol.
From table 3 and table 4, it was found that there are totally 9 positions of hydrogen bond interactions in original ligand and 18 positions of hydrogen bond interactions in new ligand. The tyrosine 355 residue which is found in the active site of the enzyme interacts 13 times at different positions in the new ligand whereas it interacts only twice at various positions in original ligand (Fig 2 and 3).

Thus docking studies using Maestro and Glide software provided information about optimum pose of new ligand in the active site of the enzyme. It was also found that the pose of the new ligand has increased glide score as compared to that of original ligand. Better the glide score better is the interaction. From the present investigations it can be inferred that analog had better interactions in the active site of the Cox – 2 enzyme and therefore it can be proposed as a new drug. Further wet lab investigation can be done to test the potency of this compound as a betterment of mankind to treat various dreadful diseases like cancer, Parkinson's disease, Alzheimer's disease, arthritis and other inflammatory diseases.

Table 1. Induced Fit Docking Score Table Of Original Ligand.

Pose	Glide energy	Glide score	Glide lipo	Glide ecoul	Glide evdw
1.	-36. 792	-8. 120	-3. 583	-5. 614	-31. 177
2.	-45. 678	-9. 152	-3. 688	-8. 53	-37. 147
3.	-43. 059	-8. 574	-3. 613	-5. 929	-37. 129
4.	-28. 558	-8. 410	-3. 613	-12. 172	-16. 385
5.	-44. 797	-9. 010	-3. 905	-10. 031	-34. 948
6.	-43. 270	-8. 547	-3. 701	-5. 320	-37. 949
7.	-38. 010	-8. 421	-3. 795	-6. 002	-32. 008
8.	-40. 318	-8. 514	-3. 756	-2. 733	-37. 584
9.	-39. 573	-6. 050	-2. 345	-2. 534	-32. 030

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Table 2: Induced fit Docking Score Table For New Ligand

Pose	Glide energy	Glide Score	Glide lipo	Glide ecoul	Glide evdw
9.	-20.400	-4.798	-1.640	-14.367	-6.033
8.	-24.808	-4.847	-1.454	-16.816	-7.992
1.	-20.965	-5.551	-2.289	-18.373	-2.591
10.	-20.722	-4.729	-1.489	-11.714	-9.009
11.	-22.374	-4.712	-1.484	-12.579	-9.795
12.	-18.755	-4.634	-1.583	-8.598	-10.157
13.	-16.855	-4.501	-1.447	-5.96	-10.90
14.	-17.688	-4.465	-1.690	-4.571	-13.11
15.	18.328	-4.446	-1.470	-6.804	-11.524
16.	-18.95	-4.060	-1.433	-9.36	-9.590
17.	-18.563	-3.624	-1.424	-4.634	-14.199
18.	-17.679	-3.487	-1.83	-4.096	-13.583
2.	-30.151	-5.482	-1.551	-22.459	-7.856
3.	-29.549	-5.372	-1.585	-19.286	-10.263
4.	-28.606	-5.346	-1.55	-20.394	-8.211
5.	-27.134	-5.324	-1.512	-23.035	-4.101
6.	-18.797	-4.921	-1.90	-14.411	-4.387
7.	-20.008	-4.901	-2.101	-11.241	-8.767
8.	-24.809	-4.848	-1.455	-16.816	-7.993
9.	-20.401	-4.798	-1.640	-14.367	-6.033

ORIGINAL LIGAND

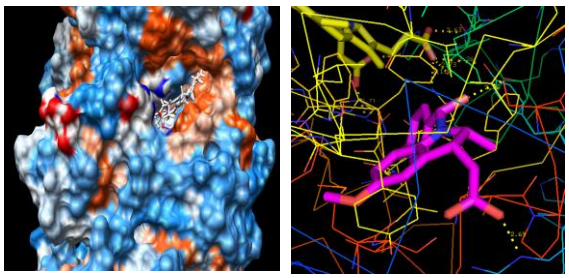

Fig.2 Original Ligand

NEW LIGAND

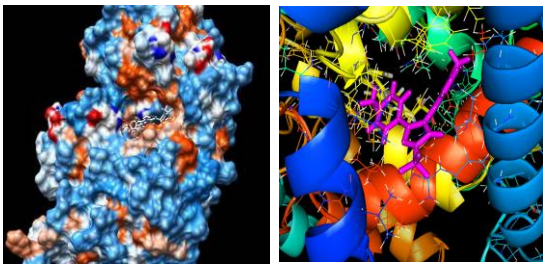

Fig. 3 New Ligand

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Table 3.Hydrogen Bond Interaction Score For Original Ligand

POSE	INTERACTIONS
01.	O—H---O(SER530, 2. 920)
01.	O—H---N(ARG 120, 3. 128)
02.	O—H---O(SER530, 2. 959)
02.	O—H---N(ARG 120, 2. 685)
03.	O—H---O(SER530, 2. 820)
03.	O—H---N(ARG 120, 3. 040)
04.	O—H---O(SER530, 2. 786)
04.	O—H---N(ARG 513, 2. 740)
04.	O—H---N(ARG 120, 3. 003)
04.	O—H---O(TYR 355, 2. 625)
05.	O—H---O(SER530, 2. 813)
05.	O—H---O(TYR 355, 2. 807)
06.	O—H---O(SER530, 2. 887)
06.	O—H---N(ARG 120, 3. 219)
07.	O—H---O(SER530, 3. 075)
07.	O—H---N(ARG 120, 2. 875)
08.	O—H---N(ARG 120, 3. 165)
08.	O—H---O(SER530, 3. 000)

Table 4: Hydrogen Bond Interaction Score for New Ligand

S.no	Interactions & binding energy	Pose
01	F---H----N(ARG 120,2.79A)	01
02	N---H----O(TYR 355,2.91A)	10
03	N---H----O(TYR 355,2.96A)	11
04	F---H----O(TYR 355,3.10A)	12
05	N---H----O(TYR 355,3.09A)	12
06	N---H----O(TYR 355, 2.98A)	14
07	N---H----N(ARG 120,3.11A)	14
08	N---H----O(TYR 355,2.77A)	15
09	N---H----O(TYR 355,3.06A)	16
10	N---H----N(ARG 120,3.01A)	17
11	N---H----N(ARG 120,2.03A)	18
12	N---H----O(TYR 355,3.76A)	02
13	N---H----O(TYR 355,2.78A)	03
14	N---H----O(TYR 355,2.85A)	04
15	F---H----N(ARG 513,2.96A)	04
16	N---H----O(TYR 355,3.06A)	05
17	F---H----N(ARG 120,2.88A)	06
18	N---H----O(TYR 355,2.93A)	08
19	N---H----O(TYR 355,2.77A)	09
20	N---H----O(TYR 355,2.98A)	13

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